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(71) Applicant: DAVID SARNOFF RESEARCH CENTER, INC. [US/US]; 201 Washington Road CN5300, Princeton, NJ 08543-5300 (US).

(72) Inventors: ZANZUCCHI, Peter, J.; 13 Jill Drive, Lawrenceville, NJ 08648 (US). CHERUKURI, Satyam, C.; 90 Cranbury Neck Road, Cranbury, NJ 08512 (US). MCBRIDE, Sterling, E.; 4214 Fieldcrest Court, Lawrenceville, NJ 08638 (US).

(74) Agent: BURKE, William, J.; David Sarnoff Research Center, Inc., 201 Washington Road CN5300, Princeton, NJ 08543-5300 (US).

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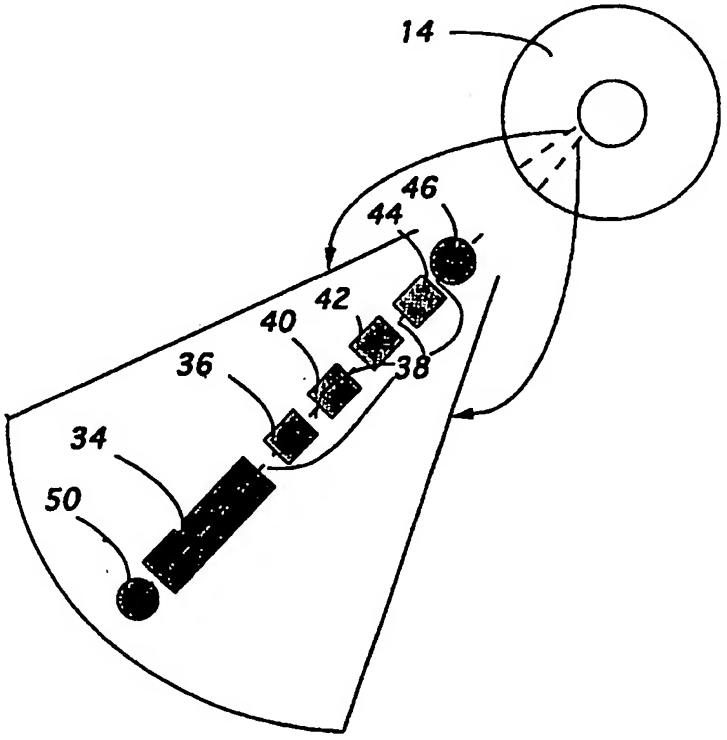
(54) Title: A PARTITIONED MICROELECTRONIC DEVICE ARRAY

(57) Abstract

A system for processing a plurality of tests or syntheses in parallel comprising a sample channel for moving samples into a microlaboratory array of a plurality of wells connected by one or more channels for the testing or synthesis of samples, a station for housing the array and an optical system comprising at least one light source and at least one light detector for measuring the samples in the array, and a means of electrically connecting said array to an apparatus capable of monitoring and controlling the flow of fluids into the array. Samples are loaded from a common loading channel into the array, processed in the wells and measurements taken by the optical system. The array can process many samples, or synthesize many compounds in parallel, reducing the time required for such processes.



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A PARTITIONED MICROELECTRONIC DEVICE ARRAY

This invention relates to a system comprising a partitioned microelectronic and fluidic array. More particularly, this invention relates to a system including an array of microelectronic and fluid transfer devices for carrying out various processes, including syntheses, screening and chemical diagnostic assays, in parallel, and method of making the array.

Traditional methods of making a homologous series of compounds, or the testing of new potential drug compounds comprising a series of like compounds, has been a slow process because each member of the series or each potential drug must be made individually and tested individually. For example, a plurality of potential drug compounds is tested by using an agent to test a plurality of materials that differ perhaps only by a single amino acid or nucleotide base, or have a different sequence of amino acids or nucleotides.

Recently the process has been improved somewhat by combining the synthesis of various compounds having potential biological activity, for example, and traditional semiconductor techniques. A semiconductor or dielectric substrate for example is coated with a biologic precursor having such amino groups with a light-sensitive protective chemical thereover, and a series of masks are placed over the substrate, each mask having an opening. A coupling agent, such as a photosensitive amino acid, is illuminated through the opening, forming a particular compound by reaction with the amino compound. Additional masks are used with different coupling agents to form an array of different peptides on the substrate which array can then be tested for biologic activity. Suitably this is done by exposure of the array to a target molecule, such as an antibody or a virus. The array is exposed to a biologic receptor having a fluorescent tag, and the whole array is incubated with the receptor. If the receptor binds to any compound in the array, the site of the fluorescent tag can be detected optically. This fluorescence data can be transmitted to a computer which can compute which compounds reacted and the degree of reaction. This technique permits the synthesis and testing of thousands of compounds in days rather than in weeks or even months.

However, the synthesis of each coupling reaction is not always complete, and the yield decreases as the length of the biopolymer increases. The process of aligning a plurality f masks and forming openings in the masks in sequence requires careful alignment and takes time.

The above synthesis is made p ssible by two other recent technical developments that all w various manipulations and reactions on a planar

surface. One is the detection and analysis of DNA fragments and their identification by reaction with specific compounds. Probes, RNA and DNA fragments can be resolved, labeled and detected by high sensitivity sensors. The presence or absence of DNA fragments can identify diseased cells for example.

Another step forward is the ability to separate materials in a microchannel, and the ability to move fluids through such microchannels. This is made possible by use of various electro-kinetic processes such as electrophoresis or electro-osmosis. Fluids may be propelled through very small channels by electro-osmotic forces. An electro-osmotic force is built up in the channel via surface charge buildup by means of an external voltage that can "repel" fluid and cause flow. This surface charge and external voltage produces an electro-kinetic current that results in fluid flow along the channel. Such electro-kinetic processes are the basis for a device is described by Pace in US Patent 4,908,112 for example.

Thus real progress has been made using electrophoresis and/or electro-osmosis to move very small amounts of materials along microchannels. Such movement can be used for synthesizing very small samples of potential drug compounds in an array and testing very small amounts of materials for bioactivity. Further progress in fully automating the fluidic processes will result in the synthesis and testing of vast numbers of compounds for bioactivity of all types, which information can be made available for future drug selection and will greatly reduce the time and expense of such testing.

The system of the invention comprises a device array of micron sized wells and connecting channels in a substrate that interfaces with a station for dispensing fluids to and collecting fluids from, the array, and for performing electro-optic measurements of material in the wells. The station is also connected to control apparatus means to control the fluid flow to the channels and wells and to collect measurement data from the substrate. The above elements are interdependent and together can perform a variety of tasks in parallel.

The individual wells of the array and their sequence can be varied depending on the synthesis or analysis to be performed. Thus the function of the arrays can be readily changed, with only the additional need to choose suitable interface means for monitoring and c ntrolling the flow of fluids to the particular array being used and th test or synthesis to be performed.

In one embodiment the above system can be used to perform various clinical diagnostics, such as assays for DNA in parallel, using the known

protocols of the polymerase chain reaction (PCR), primers and probe technology for DNA assay. In another embodiment the above system can be used for immunoassays for antibodies or antigens in parallel for screening purposes. In still other embodiments, the synthesis of a series of chemical compounds, or a series of peptides or oligonucleotides, can be performed in parallel. Each well in the array is designed so to accomplish a selected task in appropriate modules on a substrate, each module containing the number of wells required to complete each task. The wells are connected to each other, to a sample source and to a source of reagent fluids by means of connecting microchannels. This capability permits broad based clinical assays for disease not possible by sequential assay, permits improvement in statistics of broad based clinical assays such as screening of antibodies because of the parallelism, permits a reduction in costs and an improvement in the speed of testing, and permits improved patient treatments for rapidly advancing disease.

The array is formed in a suitable dielectric substrate and the channels and wells are formed therein using maskless semiconductor patterning techniques. The station and control means, such as a computer, use existing technology that includes commercially available apparatus.

The present device array uses active processing to move fluids across the array, reducing the time required for synthesis and screening. Further, large biopolymers of all types can be synthesized and processed while maintaining high purity of the synthesized compounds. The present microlaboratory arrays may be fully automated, enabling the rapid transfer of samples, precursors and other movement of fluids into the array, from one well to another well, and to enable the measurement of assays and the complete control of processing parameters such as temperature control.

T he teachings of the present invention can be readily understood by considering the following detailed description in conjunction with the accompanying drawing, in which:

Fig. 1A is an exploded schematic diagram of the parts of the system of the invention adapted for performing clinical assays.

Fig. 1B is a top view of a substrate of the invention illustrating a single module formed therein.

Fig. 2 is an exploded top view of an illustrative module of the invention.

Fig. 3 is an exploded cross sectional view of th optical transmission and detection system f the invention.

Fig. 4A is a cross sectional view of a well embodiment of a

microlaboratory disc of the invention.

Fig. 4B is a cross sectional view of another well embodiment of a microlaboratory disc of the invention.

Fig. 5A is a cross sectional view of a portion of a module of a microlaboratory disc illustrating devices in typical wells.

Fig. 5B is a cross sectional view of a portion of a module of a microlaboratory disc covered with a cover plate.

Fig. 6A is a cross sectional view of a microlaboratory disc illustrating additional wells having preformed devices therein together with an optical system interface.

Figs. 6B and 6C illustrate a valve situate in a channel adjacent to a well in the open and closed positions respectively.

Fig. 7A is an exploded schematic view of another embodiment of the present invention adapted to perform immunological assays.

Fig. 7B is a top view illustrating a module on the microlaboratory disc of Fig. 7A.

Fig. 7C is a cross sectional view of a control means for moving fluids in the channels and from one well to another.

Fig. 8 is a schematic view of another embodiment of a microlaboratory array suitable for carrying out the parallel synthesis of proteins and oligonucleotides.

Fig. 9 is a schematic view illustrating a modified station for the system of Fig. 8.

Fig. 10 is a schematic view of a further embodiment of a microlaboratory array suitable for carrying out the synthesis of a large number of small molecules in parallel.

Figs. 11A, 11B and 11C are cross sectional views illustrating the steps needed to form cross over channels in the substrate.

Fig. 11D is a top view of a cross-over channel in the substrate.

Fig. 12 is a top view of a channel "gate" electrode to control flow in a channel by electro-osmosis.

The invention will be first described by reference to Fig. 1A, which illustrates the parts of an illustrative system of the invention configured to perform DNA screening diagnostics.

The system of Fig. 1A includes a computer 10, electrically connected via line 11 to peripheral apparatus, such as a modem or printer 12, which computer 10 is programmed to give instructions to a microlaboratory disc 14 and to record test results obtained therefrom. The computer 10 is electrically

connected to a station 16 via line 15. The station 16 includes a microlaboratory disc support 18, support tubing 20 for loading test materials and reagents onto the microlaboratory disc 14, pumps 22 for moving fluids to particular destinations on the disk 14, one or more light sources 24, an optical fiber 25 and one or more light detectors 26. The optical fiber 25 is operative to transmit light from the light source 24 to the detector 26. One or more containers 28 for waste fluids and the like are also housed in the station 16.

A small volume of a fluid to be tested, such as a whole blood or other DNA-containing fluid sample, is loaded into the system via a loading system 30. The system 30 may house one or more capillary tubes 32 containing a sample which is connected to a loading capillary channel 34 etched into the surface of the microlaboratory disc 14. The loading capillary tube 32 is inserted into the capillary loading channel 34 horizontally. The sample is moved into the capillary loading channel 34 and the fluid sample is then moved to a first well 36 on the disc 14. Alternately, a loading channel 50 for vertical insertion into the loading channel 34 can also be used to load the sample. Fig. 1B and Fig. 2 illustrate a particular module comprising a plurality of wells 36, 40, 42 and 44 connected by a channel 38, shown by a dashed line, on a microlaboratory disc 14 of the invention. Each module is connected to a well 46 that collects excess or waste fluids from the respective module. These waste fluids are collected and moved to the waste containers 28 in the station 16.

The sample is treated sequentially in a series of wells including first well 36. For example, in the first well 36 the whole blood sample is transferred from the capillary loading channel 34, filtered and lysed to separate the white and red corpuscles, and the DNA is isolated from the white blood cells. The DNA sample is then moved out of the first well 36 through a connecting channel 38 that connects all of the wells of a single module, and into a second well 40. In the second well 40 the DNA is separated into single strains and amplified using the well known PCR method. The treated sample is then moved out of the second well 40 via the connecting channel 38 and into a third well 42. In the third well 42 the DNA is assayed by known probe hybridization techniques. The DNA assay is detected and evaluated in the fourth well 44. Thus the determination of DNA in a particular blood sample is performed in a series of four wells connected by a channel. Lastly excess reagents and the like are collected in th fifth well 46 that is common to all f the modules in the microlaboratory array 14, and is transferred into the waste collection system 28 f the stati n 16.

The combination of a loading channel 34 or 50, the wells 36, 40, 42, 44 and 46 and the connecting channel 38 make up one module 48 on the test microlaboratory disc 14. A single module on a microlaboratory disc 14 is shown in top view in Fig. 1B within the dashed lines. As will be explained hereinbelow, a plurality of modules are formed in the microlaboratory disc 14 so that tests can be performed on a large number of the modules 48 in parallel.

The particular sequence of channels and wells of the module shown in Figs. 1A, 1B and Fig. 2 lends itself to the detection of pathogenic bacteria in blood or other DNA-containing fluids using the DNA assay protocols of Greisen et al, see "PCR Primers and Probes for the 16S rRNA Gene of Most Species of Pathogenic Bacteria, Including Bacteria Found in Cerebrospinal Fluid", J. Clinical Microbiology 32 (2), pp335-351 (1994). Since the disc 14 can be arranged to form a plurality of up to 1500 parallel modules, broad DNA screening can be conducted in parallel that might not be possible using a system of sequential assays. Since a large array of parallel tests can be done at the same time, the time required to obtain meaningful results is also reduced, and consequently costs are reduced. Further, the statistics of broad based screening can be improved. In addition the system of the invention provides the ability to detect mutant DNA such as is found in oncological diseases.

The station 16 includes a fiber optic assembly 25 and one or more light sources 24 and one or more detectors 26 (not shown) that address the system loading channel 34 or 50 and measures the transmittance or absorbance of material in the channel 34 or 50 and the first well 36, such as a blood or other fluid sample. The fiber optic assembly 25 can verify the presence or absence of materials in the channel 34 or 50 or the well 36, and quantify their amounts by transmitting the measurement data to the computer 10. Suitable lasers and photodetectors are available commercially. Fiber optic adaptors to support the optical fiber are commercially available. These adaptors may also include a lens for efficient transfer of light from the light source into the fiber.

Preselected substrates, designed either for modular parallel or array processing, can be placed on the substrate holder 18 in the station 16. After suitable modification of the software in the computer 10 or choice of other driving means able to m nitor a particular sequence f steps for contr l of reagents t carry out the processes in each module of the microlaboratory 14, entirely different tests and processes can be carried out in the system of the inventi n. Thus the microlab ratory array 14 is individually designed for a

variety of assay, measurement and synthesis tasks, and only the module configuration and the driving means needs to be changed in the system when a different task is to be performed.

A circuit of thin film transistors (not shown) can be formed on the front or back of the glass or other substrate 14 to provide power to the wells via leads and electrodes explained further hereinafter, and to connect them with the driving means such as the computer 10, so as to move liquids along the array. Pins can also be formed in the glass substrate which are addressable by logic circuits on the support 18 that are connected to the computer 10 for example. These transistors and logic circuits will also be in contact with the substrate 14 on the support 18 to provide an interface between the microlaboratory disc 14 and the computer 10.

The system loader 30 is situated outside of the station 16 and may be connected to a loading capillary tube 32, as shown in Fig. 3, suitably having an inner diameter of about 200 microns and an outer diameter of about 600-700 microns. The capillary tube 32 can be pretreated to eliminate surface adsorption of proteins and related bio materials in known manner, for example similar to the methods disclosed by Cobb, "Electrophoretic Separations of Proteins in Capillaries with Hydrolytically Stable Surface Structures", Anal. Chem. 62, (1990) pp 2478-2483. All of the sample material is loaded into the system via the system loader 30. The system capillary tube 32 can be loaded horizontally directly into the microlaboratory disk 14 via the loading channel 34. The sample is identified, as with a bar code or other high density code affixed to the loading capillary tube 32 as by its position on the disc array. As the sample loading tube 32 is inserted into the loading channel 34, a sealant, which can be adhered to the edge of the capillary sample tube 32 or to the loading channel 34, seals the capillary tube 32 to the channel 34. Alternately, the sample capillary tube 32 can be inserted into a separate loading channel 50 vertically, which permits a smaller loading channel to be used, but the sample may enter into the connecting channel 38 through force of gravity rather than via a controlled pump feed from the pumps 22 in the station 16. This alternate configuration 50 is also shown in Figs. 1-3.

The heart of the present system is the parallel modular microlaboratory disc 14, shown in a side view in Fig. 1A and in a top view in Fig. 1B. Figs. 1B and 2 illustrate a single module on a microlaboratory disc 14, which is suitably about 8.5 cm in diameter.

Each module of the microlaboratory array is made up f one or m re wells connected by a channel, in turn connected to the loading capillary tube.

The microlaboratory disc 14 itself can be made from glass, fused silica, quartz or a silicon wafer for example, suitably about 1 millimeter thick, that is able to be finely and controllably etched using semiconductor techniques and a suitable etchant such as HF. High quality glasses such as a high melting borosilicate glass or a fused silica, will be preferred for their UV transmission properties when any of the processes or measurements carried out in the wells or channels use light based technologies. The module 48 illustrated in Fig. 1B comprises four connecting wells, but this is by way of example only, and more or fewer wells can be present depending on the tests or syntheses to be performed in each module, and the number of steps required to perform them, as will be further described hereinbelow.

The desired number of wells are etched into the microlaboratory disk sufficient to perform the sequence of steps to be used for testing or synthesis for that particular microlaboratory disc. All of the wells in each module are connected together via one or more channels. The modular design permits efficient placement of each module on the disc substrate. When only a few wells are required for each module, two modules can be formed in each radial slice of the disc (not shown), thereby doubling the number of modules that can be etched into a single microlaboratory disk 14. Thus for an 8.5 cm diameter disk, an array of 267 single modules or 534 double modules can be readily made. When the sample is inserted vertically into the loading channel 50, which takes up less area of the substrate, additional modules can be accommodated on a single microlaboratory disc, and up to 1500 modules can be formed on a single microlaboratory disk 14. Each of the modules is connected to the system loading system 30, thus permitting parallel processing in all of the modules.

Excess fluids and waste materials for all of the modules are passed into a center well 46. A plurality of wells 46 may be connected to each other by means of a common channel 47 (Fig. 1B). A sealed tubing on the back of the substrate 14 provides an exit channel for these excess fluids that can be passed into the waste container 28 in the station 16. Thus only one exit channel 47 needs to be provided for each microlaboratory array.

The wells of the microlaboratory disc 14 can be made by the following procedure. A glass substrate disc 14 is coated sequentially on both sides with a thin chromium layer and a gold film about 1000 angstroms thick in kn wn manner, as by evaporation or chemical vapor depositi n (CVD), to protect the disc from subsequent etchants. A two micron layer f a photoresist, such as Dynakem EPA of Hoechst-Celanese Corp. is spun n and the ph toresist is

exposed, either using a mask or using square or rectangular images, suitably using an MRS 4500 panel stepper of MRS Technology, Inc. After developing the resist to form openings therein, and baking the resist to remove the solvent, the gold layer in the openings is etched away using a standard etch of 4 grams of KI and 1 gram of iodine (I_2) in 25 ml of water. The underlying chromium layer is then separately etched using an acid chromium etch, such as KTI Chrome Etch of KTI Chemicals, Inc. The glass substrate is then etched in an ultrasonic bath of HF-HNO_3-H_2O in a ratio by volume of 14:20:66. The use of this etchant in an ultrasonic bath produces vertical sidewalls for the various wells. Etching of the wells is continued until the desired depth of the well is obtained, suitably about 200-750 microns deep, depending upon the functions to be performed in the well.

The connecting channel 38, suitably about 200 microns in width and 50-100 microns deep, is then etched between the wells, when the remaining photoresist and chromium-gold layer is removed.

Fluid material may be transmitted to the various wells from the first well 36 or from the loading channel 34 by various methods. An external mechanical pumping system 22 that can deliver fluid in reproducible and accurately controlled amounts and that can maintain the purity and sterility of test liquids can be employed. The 205U multichannel cassette pump available from Watson-Marlow, Inc is a suitable pump. Alternatively, miniaturized mechanical pumps, based on microelectromechanical systems (MEMS) can be used. These miniature pumps may be internal or external to each well 36, 40, 42 and 44. Such pumps have been reported by Shoji et al, "Fabrication of a Micropump for Integrated Chemical Analyzing Systems", Electronics and Communications in Japan, Part 2, 70, pp52-59 (1989). Other suitable pumping means to move fluids through microchannels include electrokinetic pumps as reported by Dasgupta et al, see "Electroosmosis: A Reliable Fluid Propulsion System for Flow Injection Analysis", Anal. Chem. 66, pp1792-1798 (1994), or electrophoresis methods, which require inert metal electrodes, can also be used. In the latter case, a gold electrode can be deposited in the various wells of a module, as shown for example as metal layer 54 in the bottom of the first well 36, as shown in Fig. 4A. The electrode 54 is connected via leads 55 to a circuit or other electrical connection 56.

When large amounts of fluid need to be passed through the connecting channel 38, such as diluents, buffer solutions, rinse solutions and the like, an external mechanical pump 22 in the station 16 will be used to pass these solutions through the channel 38 into the desired well. When smaller amounts

of material are required, an on-disk internal pumping system can be used for greater efficiency. Further, prepackaged chemicals sealed in plastic release containers can be deposited in the various wells for particular tests, as required.

In the case where the temperature of a particular well is to be monitored or changed, a means of heating or cooling the well is built into the well, as will be further explained below with reference to Fig. 4B.

The first well 36 in this example is the sample preparation well. A thin film of a suitable metal oxide 57, such as tin oxide or indium tin oxide, is deposited onto the well material and is connected by means of an electrically conductive metal connection 58 to the end or outer edge of the well 36. The tin oxide coating 57 serves as a heater element for the well 36. The well 36 also has a surface bimetal film 59 and leads 60, suitably made of chromel-alumel alloys, forming a thermocouple to measure the temperature in the well when a source of current is applied to the tin oxide coating 57 and to the leads 58. A voltage applied to the well 36 via electrodes 56 deposited on the backside as shown, or preferably on the frontside of the microlaboratory disc 14, regulates the temperature in the well. The amount of current applied can be regulated by the computer 10 in response to the temperature measured through the leads 60.

The first well 36 of the present module 48 also contains a blood affinity binding material, (not shown) such as Leukosorb^{TM} available from Pall Co. and commercially available solutions to lyse the red and white blood cells of the sample and to carry the DNA from the first well 36.

Thus the devices and certain reagents needed for each well will be preformed and loaded into the well or directly pumped into the well from a common source. Fluids can be preloaded into the well either from a reservoir in the station 16 leading to the loading capillary tube 32 connected to the microlaboratory disc 14, or a reservoir and channel into the well may be formed adjacent to the well in which the fluids will be used.

Additional devices can be built into the wells. For example, a means of stirring reactants can also be built into a well using alternating fields. For example, two or more electrodes can be evaporated into each side of a well and connected to external leads, in turn connected to a source of alternating current. The alternating fields will provide reversible magnetic fields to move paramagnetic particles in the wells and to cause, as w ll, fluid movement in the well.

As sh wn in Fig. 5A, paramagnetic beads 61 are deposited in the

second well 40 of the present module 48, to bind DNA material and to move the DNA to the succeeding wells for amplification, detection and assay. These beads are commercially available for PCR protocols from Bang Laboratories of Carmel,IN for example. The well 40 is also connected by means of leads 66 to the electrodes 56.

When all of the wells have been prepared and loaded, a glass cover plate 63 is affixed to the microlaboratory disc 14, as shown in Fig. 5B, to complete a capillary structure for the connecting channel 38 and to ensure that fluids in the wells do not evaporate. The cover plate 63 can be made of the same or different material as the microlaboratory disc, but the thermal coefficient of expansion of the cover plate 63 and the material used to make the microlaboratory disc 14 must be similar. The sealing temperature required to seal the cover plate 63 to the disc 14 must also be below the flow temperature of the disk material to prevent distortion of the etched channels and wells. The use of electric fields to seal the cover plate to the disk is suitable because sealing will take place at a lower temperature than the flow temperature, e.g., about 700°C or less, which is well below the flow temperature of silicon, about 1400°C, or of Corning 7059 glass (about 844°C), available from Corning Glass Co., for example.

The invention will be further explained in terms of examples that describe different modules for performing different processes, such as assays and syntheses, simultaneously and in parallel. However, the invention is not meant to be limited to the details described therein and additional processes and syntheses can be readily performed by appropriate changes to the number of wells in a module, the devices formed in or adjacent to the wells, the number and type of devices needed to pass fluids into and out of the wells, the number and type of processing-type devices built onto the disc other than the modules, and the like.

Example 1 DNA Analysis

The sample fluid is passed into the loading channel 34 or the loading channel 50 as explained above. The sample then moves by application of an electric field or is moved by a pump 22 into the first well 36 through the channel 38, where the separation of the sample, e.g., filtration, lysation and DNA separation, takes place. The first well 36 is fitted with a means of heating and temperature control, as shown in more detail in Fig. 5. A layer of tin oxide 57 is first deposited in th well 36 by CVD. A bilayer film 59 is d posited over the tin oxide film 57 in the well 36, and a metal connection 60 is d posited along a sidewall of the well. Electrodes 56 and 60 are formed on the

backside of the microlaboratory disc 14, and leads 58 and 60 connect the thermocouple 59 to the external contacts 56. The current in the leads 58 and the voltage from the leads 60 are monitored and controlled by the computer 10.

The well 36 is also preloaded or post loaded with a blood affinity binding material such as Leukosorb^{TM} media from Pall BioSupport Div. Other commercially available materials can also be used as desired for the same purpose. The amount of Leukosorb B employed depends on the area in the first well 36. The Leukosorb B filters the blood cells from the blood serum sample. A known buffer solution is then passed into the first well 36 by means of the channel 38 in an amount sufficient to assist in lysing and washing the red corpuscles in the sample. A white corpuscle lysis buffer solution, for example a solution of 50 millimoles of KCl, 10 millimoles of Tris HCl having a pH of 8.3, 2.5 millimoles of $MgCl_2$, 0.45% Nonidet P40 and 60 ml of Proteinase K, is then passed into the first well 36 via the channel 38 in an amount sufficient to lyse the white corpuscles. The first well 36 is then heated to 55°C for fifteen minutes to cause the desired reaction. The cell was then heated to 100°C for about 10 minutes to inactivate the proteinase K following known procedures.

Because of the high temperatures required in the last two steps, a significant vapor pressure may develop in the first well 36, causing a back pressure in both directions - back toward the sample loading channel 34 and forward to the succeeding second well 40. Thus preformed valves 62 and 63 as shown in Fig. 6A, formed of bimetallic materials as described by Jerman et al, "Understanding Microvalve Technology", Sensors, September 1994 pp 26-36 are preloaded into the channel 38. These materials have a thermal expansion mismatch. When the temperature in the well 36 is low, the ball valve 62 is in its normal position permitting free flow of fluids into the well 36, see the arrow shown in Fig. 6B. As the temperature in the well 36 increases, the ball valve 62 moves to a cooler position, as shown in Fig. 6C, blocking the channel 38 and isolating the well 36 from the channel 38, thereby preventing fluids from passing into and out of the first well 36. By proper placement of the well heater 57, the temperature gradient from the heated well 36 will be sufficient to open and close the valves 62, 63, thus eliminating the need for separate electrical, thermal or mechanical control f the valves 62, 63.

However, other means may be employed as desired for closer control of the temperature and pressure in the well 36. A simple design comprises a small micron size sphere 64, such as on made of quartz or

polytetrafluoroethylene polymer for example, which may be confined in the channel 38 to act as a conventional check valve, i.e., to prevent backward flow into the channel 38. However, there is no check on the flow forward into the succeeding well 42 with this design, which may be disadvantageous for certain applications and combinations of sequential wells. This check valve 64 can be implemented as an insulating or magnetic sphere, and by application of externally applied electrostatic or magnetic fields, cause the valve to open or close. Such a design will permit closure of the ball valve 64 on demand, permitting control of liquids both forward and backward in the channel 38. Such a design may be particularly advantageous when a synthesis procedure is carried out on the microlaboratory disc 14.

Still referring to Fig. 6A, the prepared and treated blood sample is then transferred to the second well 40 for PCR amplification of the obtained DNA sample from the first well 36. This well 40 contains a layer of paramagnetic beads 61 to bind with the DNA material moved into the well 40. A minimum of about 10 nanograms of DNA are required for this assay. The amount actually in the well 40 can be determined by the electrooptic light source 24 and detector 26 located in the station 16. An optical fiber 25 of the light source 24 is connected to the well 40, and can be an optical fiber 25 of 200, 400 or 600 micron diameter to provide 260 nm radiation for the detection of DNA in this example. Optical fibers transmitting at 250-260 nm are commercially available. The optical fiber 25 of Fig. 6A which is connected to the detector 26 collects and transmits the light measured in the well 40 to an ultraviolet sensitive silicon photodetector 26 having good stability. Suitable detectors include thermoelectrically cooled UV enhanced silicon photodetectors, or miniaturized UV sensitive photomultipliers which are also commercially available. The photomultiplier-type detector has greater sensitivity. The absorbance of DNA is known and is set forth in the Table below.

TABLE

Species	Absorbance at 260 nm	Concentration mg/ml
Double stranded DNA	1	50
Single stranded DNA	1	33
Single stranded RNA	1	40

A well known means of assaying DNA is the hybridization technique. Th third well 42 is used for this purp s . The well 42 will be fitted by hybridization probes which are unique to the source of DNA and its diagnostic scope. The desired probes may be directly synthesized in the well 42 or

commercial sources can be used.

The method most suitable for the microlaboratory array disc 14 for assaying DNA is the use of a non-radioactive tag, which can contain a fluorescent dye, for example for the Phycobiliprotein, or other class of protein. These materials absorb light above 600 nm. A compact solid state laser 24 emitting in the 600-650 nm range situate in the station 16 can be used to produce fluorescence in the tag. The probe in this example will emit above the exciting wavelength of 600 nm, i.e., in the 600-800 nm range. The Phycobiliprotein fluorescent tag agents are commercially available, e.g., Cy_5^{TM}, from Jackson Immuno-Research Labs. Inc of West Grove PA. The fluorescence will be detected by means of the detector 26 also in the station 16. A filter 68 will also be inserted between the photomultiplier photodetector 26 and the laser 24 as shown in Fig. 6 to block the laser radiation.

A suitable detector for the fluorescence emitted by the fluorescent tag is one having high sensitivity and optimum long term stability. Other commercially available means for detection of antibodies other than that described above can also be employed. For example, enzymes that form highly colored products, resulting in detection having excellent sensitivity, or chemiluminescent compounds can be employed and detected optically. Radioisotopes can also be used, but their use is being discouraged to prevent adverse effects on the operator of the tests, and they present disposal problems. The light transmission detection system itself is also located in the station 16.

Example 2 Immunological Assay

A second example illustrating the design and utility of a microlaboratory disc 114 is shown in Figs. 7A and 7B and is one suitable for carrying out a plurality of immunological assays, also in parallel. The station 16, sample loading channel 134, and plurality of wells 136, 140, 142 and 144 connected by a channel 138 are similar to those of Example 1, but the materials and processes carried out in the wells, and the number of wells required, are different.

To carry out an immunological assay, a blood sample or other fluid sample of about 100 nanoliters is filtered to obtain a material having sufficient antibodies so that an assay can be determined using standard methods for detection of select antibodies. The microlaboratory disc 114 is able t process 200-1000 samples in parallel.

The initial transfer f a sample to the microlaboratory array 114 is the same as for Example 1. The first well 136 is loaded with preselected bound

antibodies on the cell wall, and, by transfer of appropriate reagents and fluid flow control into each well 136 of the module 148, assays can be performed with that antibody. The flow of reagents and solvent will be controlled by the station 16, and the optical detection system 24, 25 and 26 will be set up for transmittance (absorption) measurements.

A series of different antibodies can be placed in additional wells 140, 142, 144 and 146 for sequential testing for various antibodies in successive wells, as shown in Fig. 7B. The number of wells required will be determined by the number of appropriate reagents and the number of antibodies to be tested.

A control means 171 to control the fluid flow of reagents and solvents will be enabled by element 162, which is a switch or valve that controls the flow of fluid into the channel 138 and into the first well 136. For example, a magnetic sphere can form a ball valve 162 by activation of an electric field by means of the computer 10.

As shown in Fig. 7C, a ball valve 162 is fitted into the channel 138 on both sides of the well 136. A source of current 170 is placed adjacent the channel 138 to control the flow into or out of the well 136, and the backside of the microlaboratory disc 114 will have a lead 172 that connects to the driving method employed, such as the computer 10.

Example 3 Synthesis of Proteins or Oligonucleotides

This example, shown in Fig. 8, illustrates a microlaboratory disc 214 that has a modular design that permits the synthesis of a large array of proteins or oligonucleotides in parallel. Such synthesis is a great time saver for drug testing for example, when a whole series of related compounds can be synthesized in each well of an array, and then tested for biologic activity.

For example, the synthesis of solid phase oligonucleotides in a well has been described by Hayakawa et al, "The Allylic Protection Method in Solid-Phase Oligonucleotide Synthesis. An Efficient Preparation of Solid-Anchored DNA Oligomers", J. Am. Chem. Soc., Vol 112, No. 5, 1990 pp1691-1696, in a sequence of eight steps:

Step 1: washing the well with acetonitrile (CH_3CN)

Step 2: detritylation with 3% Cl_3CCOOH - CH_2Cl_2

Step 3: washing the well with acetonitrile

Step 4: adding a coupling agent such as 0.1M phosphoramidite-CH_3CN and 0.5M (4-nitrophenyl)tetrazole-CH_3CN-THF (tetrahydrofuran)

Step 5: washing the well with acetonitrile

Step 6: capping with Ac_2O-2,6-lutidine-THF (1:1:8) and 6.5% 4-(dimethylamino)pyridine (DMAP)-THF

Step 7: oxidizing with 1.1M t-C_4H_9OOH-CH_2Cl_2

Step 8: washing the well with acetonitrile to remove excess acid.

After the above 8 steps have been carried out in a reaction well 237 using different protected monomers sequentially to build up lengths of oligomers as desired, additional reagents or solvents can be used to remove protective groups used for synthesis prior to disassociating the synthesized oligomers from their support. The various oligomers in each well can then be prepared for screening. A screening cell 274 can also be built into the well 236 as shown in Fig. 8.

In this design, as shown in Fig. 8, a plurality of modules or wells 236 are connected by means of various channels, and a complete synthesis of different compounds will be carried out in a single well 236. The additional features of this microlaboratory 214 design are in the array design which permits controllable and sequential delivery of various reagents and solvents to each of the wells in parallel. The reagents and solvents can be stored in reservoirs 276, 278, 280 and 282 built into the microlaboratory array, or they may be delivered from reservoirs external to the microlaboratory array, for example in the station 16, as desired.

Figure 8 illustrates a suitable design for a microlaboratory array wherein reservoirs 276, 278, 280, 282 for reagents and solvents are included in the substrate; and Fig. 9 is a partial schematic view of a system for synthesis wherein reservoirs 276, 279, 280 and 282 are added to the station 16, each connected to a fluid pump 22 for delivery to the wells of each module.

Each well in this array has a plurality of horizontal channels 238, 239, 241, 243, 245 entering and exiting each well 236, and a plurality of vertical channels 248, 250, 252 and 254, each of which leads to a different reservoir. The reagents and solvents move along the channels by means of gating electrodes, as shown in Fig. 7C, that move the fluid along the channels.

As shown in Fig. 8, reagents are delivered in parallel from the different reservoirs 276, 278, 280 and 282 into channels 238, 239, 241, 243 respectively. Each of these channels has a built in gating electrode 162 to monitor the flow of fluids from the different reservoirs into the wells 236 as required. In this embodiment, since all of the synthesis reactions take place in a single well 236, each well 236 is placed in an array and one or more channels lead into and out of each well 236 for delivery f reagents in parallel or serially. Thus instead of a modular design, when sequential wells are used f r the various steps of the process being carried out, an array of like wells 236 is used but a different chemical synthesis is carried ut in each well. The overall

design is no longer a plurality of modules, but an array of wells, and hundreds of compounds can be synthesized on a single microlaboratory substrate 214.

After all the synthetic steps are completed, the syntheses can be monitored or screening can be conducted by passing a sample into a screening cell 274 which can be built into the well 236.

This type of array can be used to synthesize a large number of oligomers and peptides which follow a repetitive sequence of steps as above.

Example 4 Synthesis of Small Molecules

This microlaboratory array of this example 314 is also designed to produce an array of small molecules, such as a plurality of different alkyl compounds. Since such an array depends on the delivery of a different starting material for each compound, e.g., methyl-substituted, ethyl-substituted, n-propyl-substituted, iso-propyl substituted compounds and the like, a large array of starting material reservoirs or supply sources will be needed so that a different starting material will be delivered to each well. The reactants and solvents and reaction conditions, e.g., heating, will be delivered as required, see Examples 1 and 2.

For example, the synthesis of an array of 1,4-benzodiazeprine compounds can be carried out as follows:

Step a): bind the starting material 4-[4-benzoyl-6-chloro-2-fluorenylmethoxy carbonylaminobenzoyloxy-methyl]phenoxyacetic acid onto aminomethyl resin or its equivalent as a coating material on the channel surfaces;

Step b): remove the 9-fluorenyl methoxy carbonyl (FMOC) protective group from the bound material using piperidine in DMF, supplied from station reservoir 376 into the wells 338;

Step c): couple an amino acid, alpha-N-FMOC-amino acid (fluoride) from a second reservoir 378 in the well 338;

Step d): remove the FMOC protective group by moving piperidine in DMF through the wells 338;

Step e): forming a cyclic benzodiazepine derivative with 5% acetic acid in DMF from a third reservoir 380 in wells 340;

Step f): alkylate with a lithium salt in DMF from a station reservoir 382 in wells 342 that are fitted with a control for pumps and switches, monitored by the computer 10, to deliver different alkylating agents to the microlaboratory array 314 selectively, as shown in Fig. 10. Thus the "reservoir" for the alkylating agent is in reality a source of a whole series of alkylating agents that can be delivered selectively to a particular well in the

array. The fluid delivery to the wells can be controlled by a control means 171 as shown in Fig. 7C. In this manner, a different compound is made in each well. However, since each compound requires several steps for synthesis of the various compounds, several wells may be employed to synthesize each of the compounds and this microlaboratory disc 314 may comprise a plurality of modules as well.

Another feature of the present invention is the fabrication of cross-over channels that permit high density arrangements of wells/modules in an array that can be serviced from more than one channel either to transmit fluids into the well, out of the well, or to transmit the material being treated to a succeeding well. Such cross over channels can be formed in the following manner. Referring to Fig. 11A, a substrate 314 is coated on both sides with a first chromium-gold layer 310A and 310B and a photoresist layer 312A and 312B as described above. The photoresist layer 312A is exposed and developed to form openings 313 for a first channel and the chromium layer 312A is etched away in the opening 313. The glass substrate 314 is then etched through to the chromium-gold layer 310B forming openings 315. The photoresist layers 312A and 312B are re-exposed and developed to form a second pair of openings 316 and 317 on each side of the glass substrate 314. However, the second opening 316 in the layer 312A is perpendicular to the opening 317 in the layer 312B, which is parallel to the openings 315 made originally. The opening 317 is formed adjacent to the openings 315 already formed in the glass substrate 314, as shown in Fig. 11B. The glass substrate is next etched to open a passage between the openings 315 and 317, thereby forming a continuous channel that extends through the glass substrate 314 and across beneath the second perpendicular channel 316 as shown in Fig. 11C. A cover plate 318 will be bonded to both sides of the substrate 314 to enclose the cross-over channels 315-317-315 and 316 on both sides of the substrate 314 and to complete the substrate having crossing but non-intersecting channels, as shown in Fig. 11D.

In order to move materials from the reservoirs into each well of the array, the material from the reservoir can be injected into one or more the channels 316. The material in each channel moves along the channel 316 via capillary action. However, since one or more of the reactions may require more or less time depending n th amount of material employed, or to compensate for faster or sl wer m vement of certain materials through the channels, the material flow in the channels preferably can be controlled by means of a drain electrode deposited in each well, and in each reservoir and in

the channel leading from the reservoir to the respective well. By creating a negative charge at the well, the flow along the channel will be stopped, thereby permitting control of reagent feed into each well. This is shown in Fig. 12 which is a top view which shows a drain electrode 440 deposited in the well 436, in a channel 438, and in the reservoir 442.

A desired microlaboratory array can be designed to carry out a wide variety of tests and syntheses rapidly, reliably, inexpensively and in parallel, greatly reducing costs. Further, because of the integration of the microlaboratory array and means of regulating, measuring and recording information, records of tests and screening data are reliably generated and stored. Since genetic screening may become the blood tests of the future, the ability to perform DNA testing rapidly, reliably and at low cost, will be highly advantageous.

Thus although the present invention has been described in terms of particular examples, the invention is not meant to be limited to the details given above. One skilled in the art can readily substitute different mechanical or electrical means of transferring fluids through the channels and wells, of substituting various bonding agents, starting materials and reagents to perform different assays and syntheses, and to form the wells, channels and devices in the channels and wells by alternate methods. For example, integrated circuits can also be employed to move fluids and monitor and measure wells of the arrays using conventional semiconductor techniques and materials. Semiconductor technology can also be used to form leads, electrodes and the like. Thus the invention is only to be limited by the scope of the following claims.

We Claim.

1. A system comprising

an array of wells in a dielectric substrate connected to each other by a channel, said channel connected to a fluid sample source,

a station housing a support for said array electrically connected to said array, and

an apparatus electrically connected to said station for controlling the flow of fluids to said array.

2. A system according to claim 1 wherein said station contains an optical system comprising one or more light sources and one or more light detectors for monitoring said fluids in said channel.

3. A system according to claim 2 wherein said light source and light detector monitor the amount of fluid sample transferred to said array from said fluid sample source.

4. A system according to claim 1 wherein said station further includes a fluid pump for controllably transmitting fluids to said array.

5. A system according to claim 1 wherein said sample is loaded into said array by means of a system loading channel connected to a loading capillary channel in said substrate.

6. A system according to claim 1 wherein said wells and channel are grouped in a module comprising a plurality of wells connected by a channel, said modules connected to a loading channel for passing fluids into the wells.

7. A system according to claim 6 wherein a plurality of modules are present in said substrate and said loading channel can pass fluids into said modules in parallel.

8. A microlaboratory array for parallel processing comprising a dielectric substrate having one or more modules therein, said modules comprising one or more wells connected by a channel for the passage of fluids into and out of the wells.

9. An array according to claim 8 wherein the substrate is a high melting temperature glass.

10. An array according to claim 8 wherein each module channel is connected to a single loading channel for passing fluids into said modules.

11. An array according to claim 8 wherein said array comprises modules for assaying DNA from a fluid sample.

12. An array according to claim 11 wherein a well has a c nductive layer of metal oxid deposited therein and a thin film bilayer thermocouple deposited thereover and leads in the well connected to electrodes n the substrate f r

temperature control of the well.

13. An array according to claim 11 wherein a well has electrodes deposited therein connected to external leads in turn connected to an electric field source to produce reversible fluid movement in said well.

14. A method of assaying DNA which comprises

moving a liquid blood sample into a loading channel of an array of modules connected in parallel, each module having four wells and a connecting channel,

moving said sample into a first well for filtering, lysing and separating DNA,

moving the DNA fraction to a second well wherein DNA is separated and amplified using the polymerase chain reaction method,

moving the amplified DNA to a third well for assay using probe hybridization techniques, and

moving the sample to a fourth well for optical detection and measurement of DNA in the sample.

15. A method according to claim 14 wherein a red corpuscle buffer solution and a white corpuscle buffer solution are moved sequentially into the first well.

16. A method according to claim 14 wherein during the lysing reaction the cell is heated to promote the reaction.

17. A method according to claim 16 wherein the cell is heated to inactivate the lysing material after reaction is complete.

18. A method of making a dielectric substrate array comprising

etching a loading channel in said substrate

etching a plurality of modules comprising at least one well and at least one channel into said well connected to said loading channel,

depositing at least one thin conductive layer in a well,

depositing an electrode on said substrate and connecting a lead between said conductive layer and said electrode to connect said modules electrically, and

sealing a cover plate over said substrate to complete formation of the channels.

19. A method according to claim 18 wherein said channels and wells are formed by depositing a chromium-gold layer on the surface of the substrate, flowing a photoresist thereover, exposing and devel ping said resist to form one r more penings thereon, and etching wells and channels through said opening.

20. A method of performing an immunological assay comprising

moving a liquid sample containing antibodies into a loading channel of a dielectric substrate having on a surface thereof an array comprising a loading channel connected to a channel connected in turn to a well,

moving said sample into said first well containing preselected bound antibodies on a well wall,

passing a reagent to bind the antibody in the sample to the well,

moving a solvent to remove excess reagent from the well,

measuring the transmittance of the contents of the well and comparing to the transmittance of known antibodies.

21. A method according to claim 20 wherein a plurality of wells is connected to a single channel to form a module for carrying out sequential steps in different wells.

22. A method of forming perpendicular, separate channels in a dielectric substrate comprising

depositing a first and second etch resistant metal coating to both sides of said substrate,

depositing a first photoresist layer over said first metal coating, and exposing and developing said photoresist to form one or more first openings in said layer,

etching said exposed dielectric substrate to the second metal coating,

exposing and developing said first photoresist layer so as to form an opening perpendicular to said first openings,

etching said substrate through said perpendicular opening to form a channel in said substrate,

depositing a second photoresist layer over said second metal coating,

exposing and developing said photoresist to form a second channel adjacent to said first openings,

etching said first openings to open a via to said second channel,

sealing a cover plate to both sides of said substrate.

23. A system for processing a plurality of samples in parallel using a microlaboratory array comprising:

a) a substrate microfabricated to define at least one sample channel for moving a sample into the microlaboratory array;

b) a plurality of wells; and

c) a plurality of channels for connecting said plurality of wells;

d) a support station for housing the microfabricated substrate, said support station including

i) an optical system, electrically coupled to said microfabricated

substrate having a light source and a light detector for measuring the sample; and

ii) a pump electrically coupled to said microfabricated substrate for moving the sample; and

e) a computer electrically coupled to said microfabricated substrate for monitoring and controlling the processing of said samples.

24. A system according to claim 23 wherein the wells are arranged in parallel modules so that a plurality of samples can be processed in parallel.

25. A system for synthesizing a plurality of compounds in parallel comprising a microlaboratory array including

a) a substrate including a sample channel for moving a sample into the microlaboratory array;

b) a plurality of wells;

c) a plurality of channels for connecting said wells;

d) a station for housing the microlaboratory array, said station including

i) optical means for detecting and measuring the sample;

ii) pump means for pumping the sample through the array; and

iii) control means electrically connected to said substrate for monitoring and controlling synthesis of the plurality of compounds.

26. A system according to claim 25 wherein the wells are arranged in parallel modules so that a plurality of syntheses can be performed in parallel.

27. A system comprising a device array of wells and connecting channels electrically connected to a station for dispensing fluids to and collecting fluids from the array, said station including electro-optic means for performing electro-optic measurements, and said station electrically connected to control means for controlling the amount of fluid dispensed to the array.

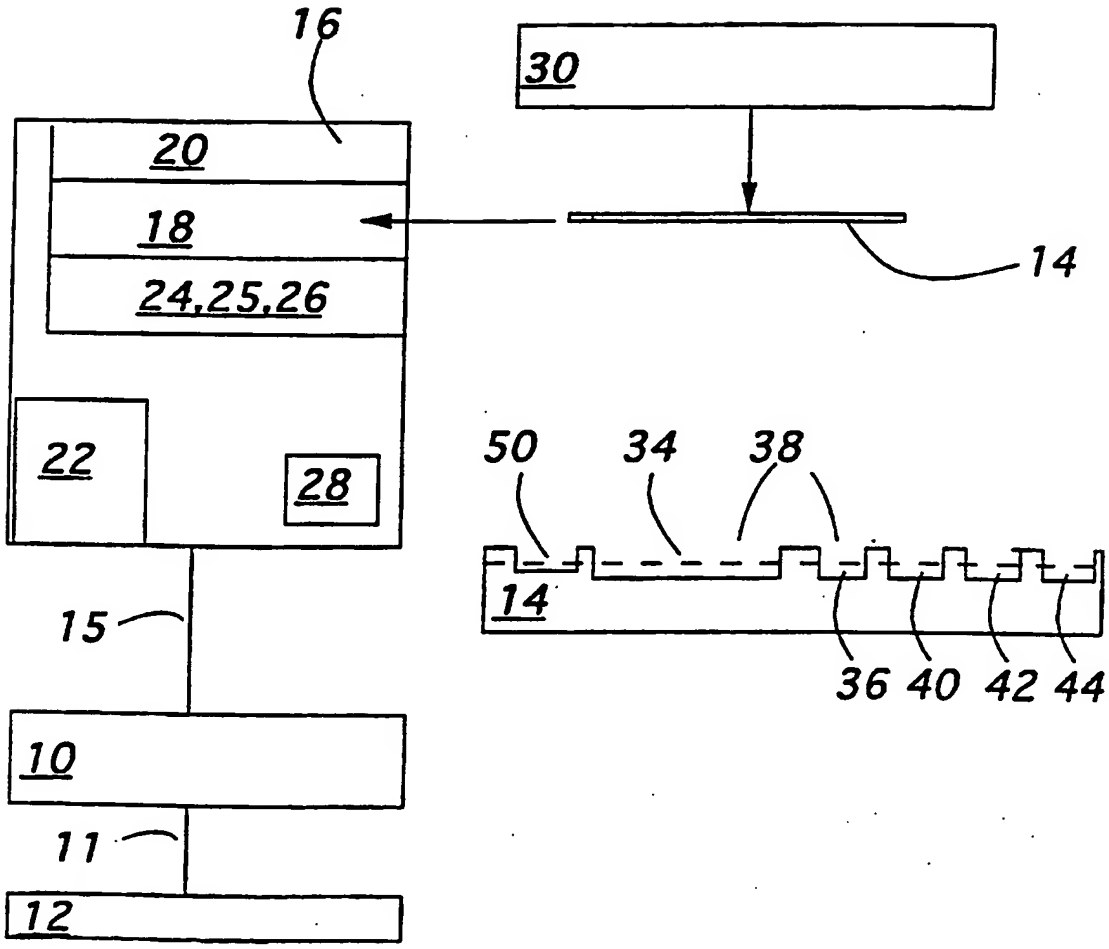

Fig. 1A

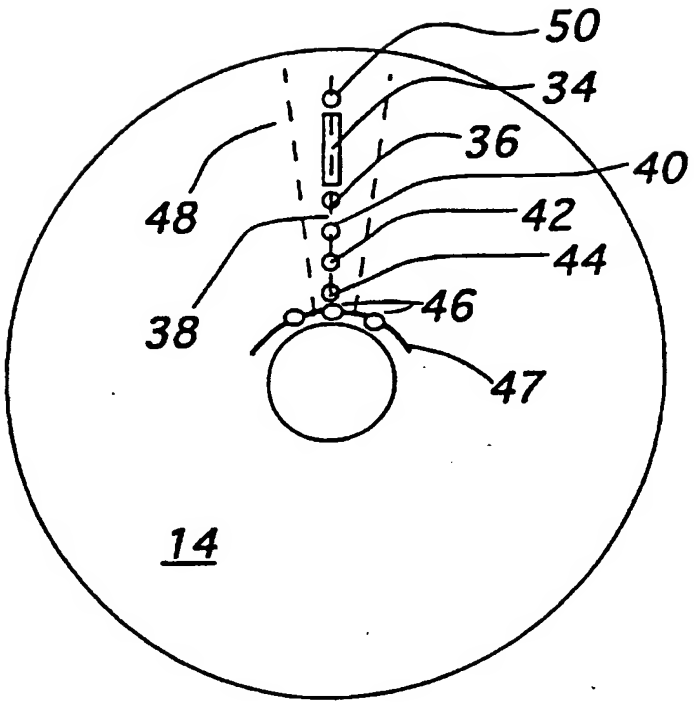

Fig. 1B

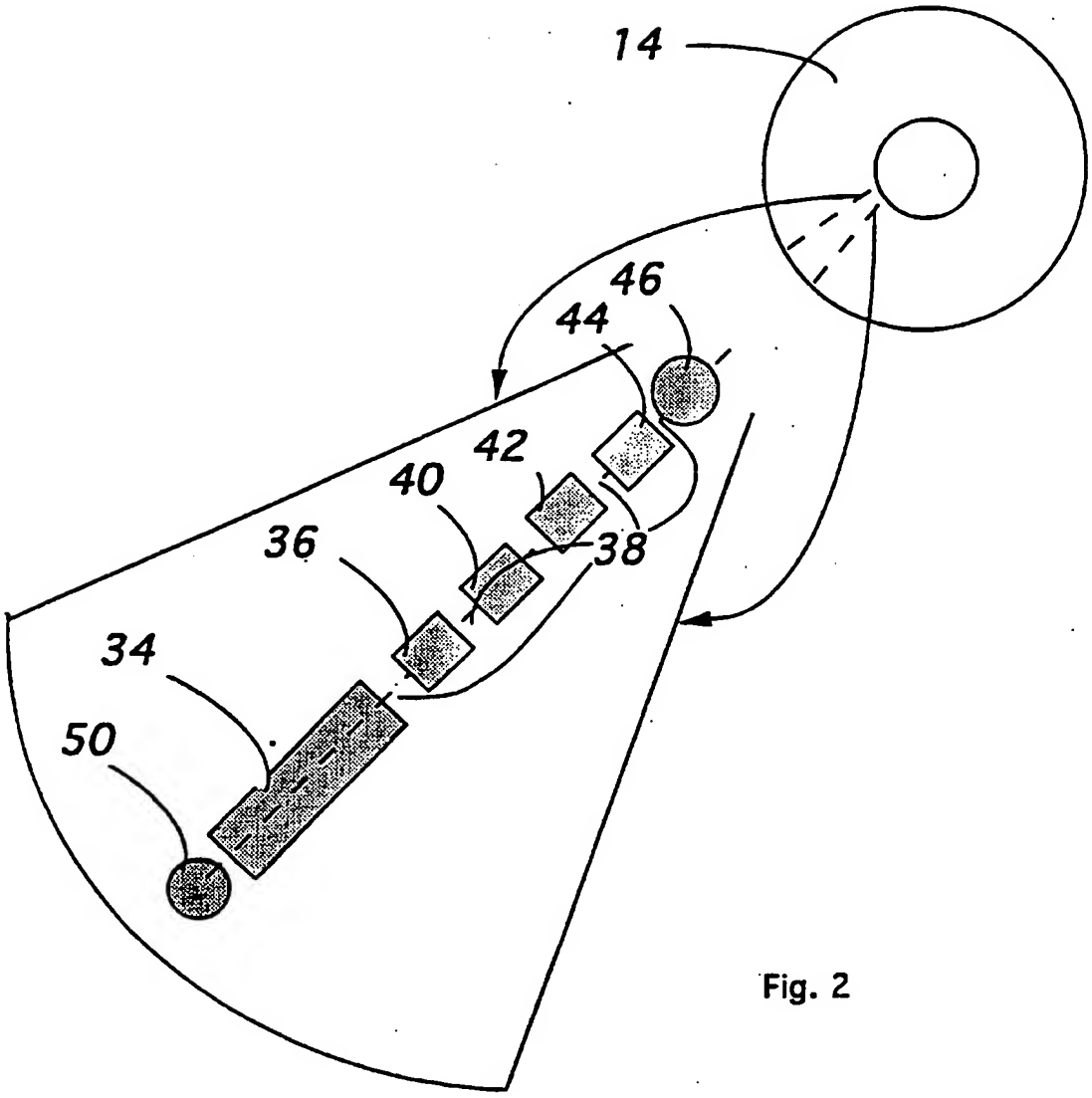

Fig. 2

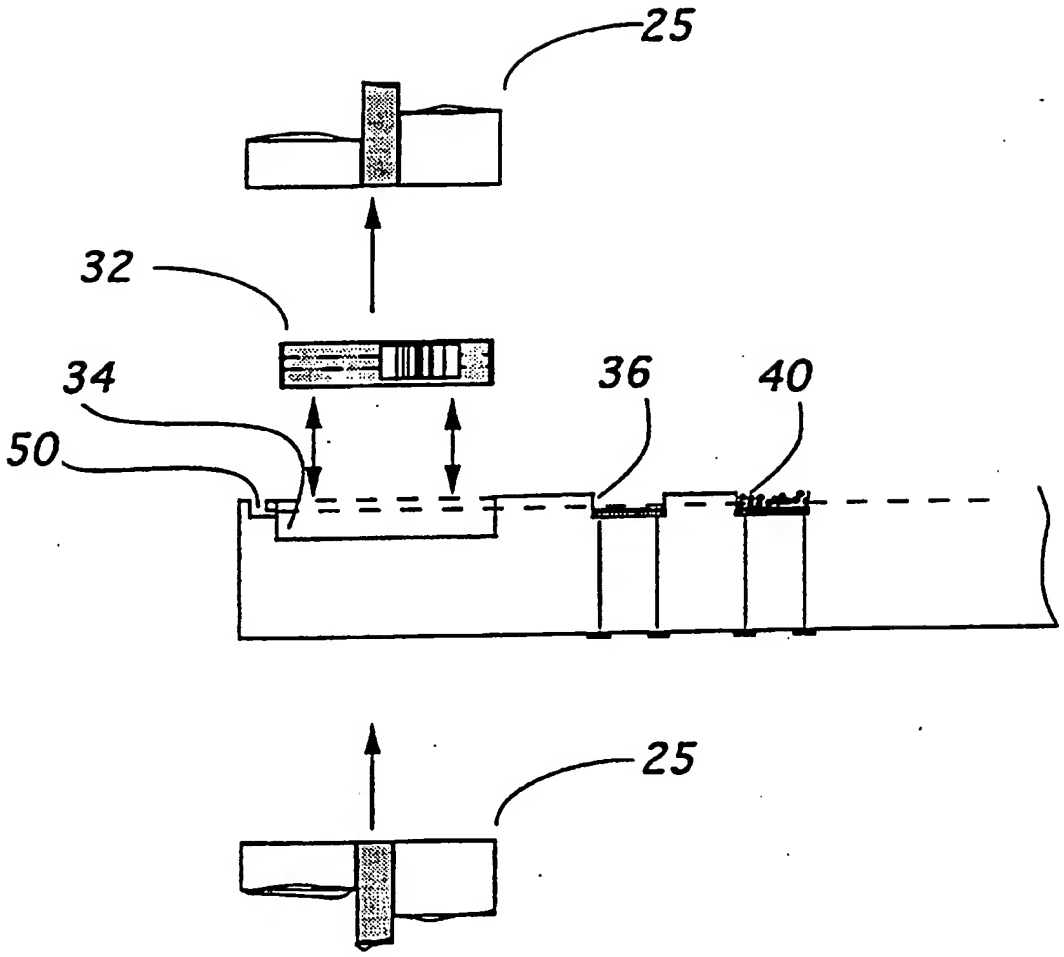

Fig. 3

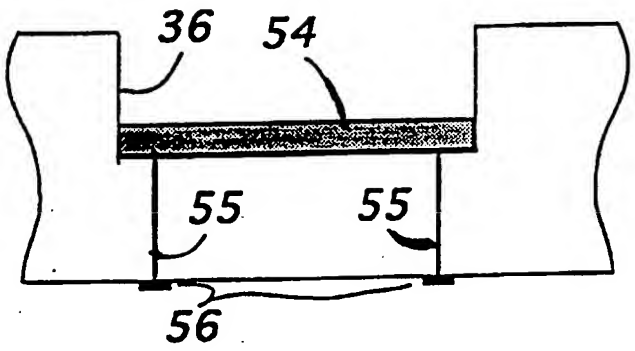

Fig. 4A

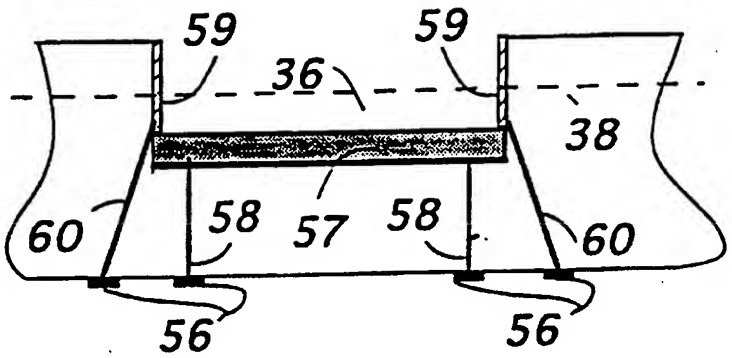

Fig. 4B

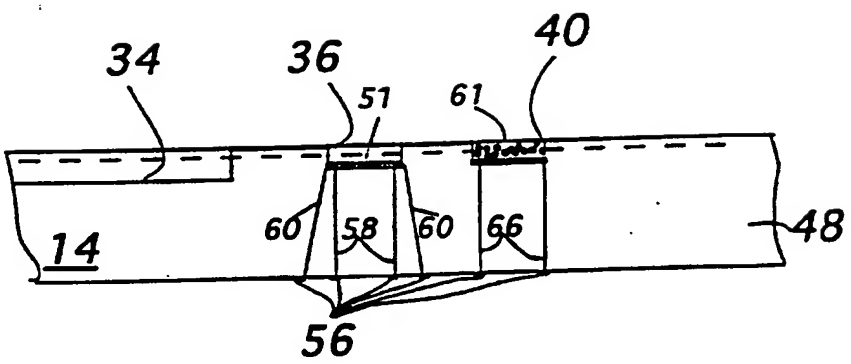

Fig. 5A

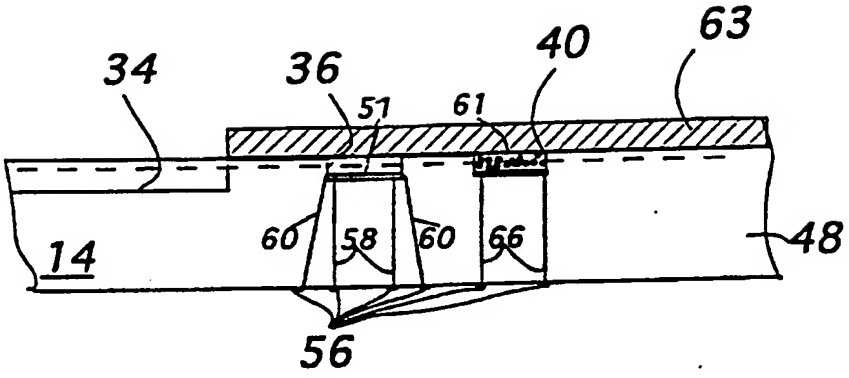

Fig 5B

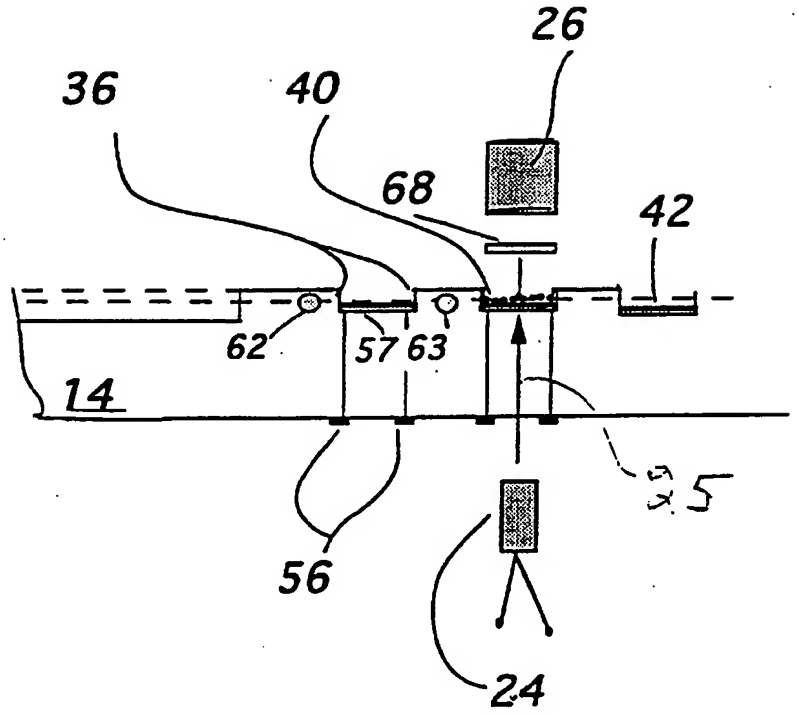

FIG. 6A

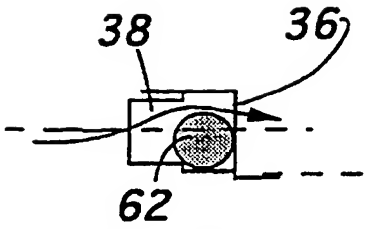

FIG. 6B

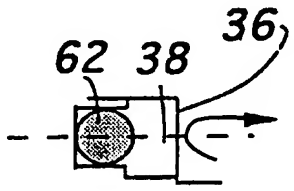

FIG. 6C

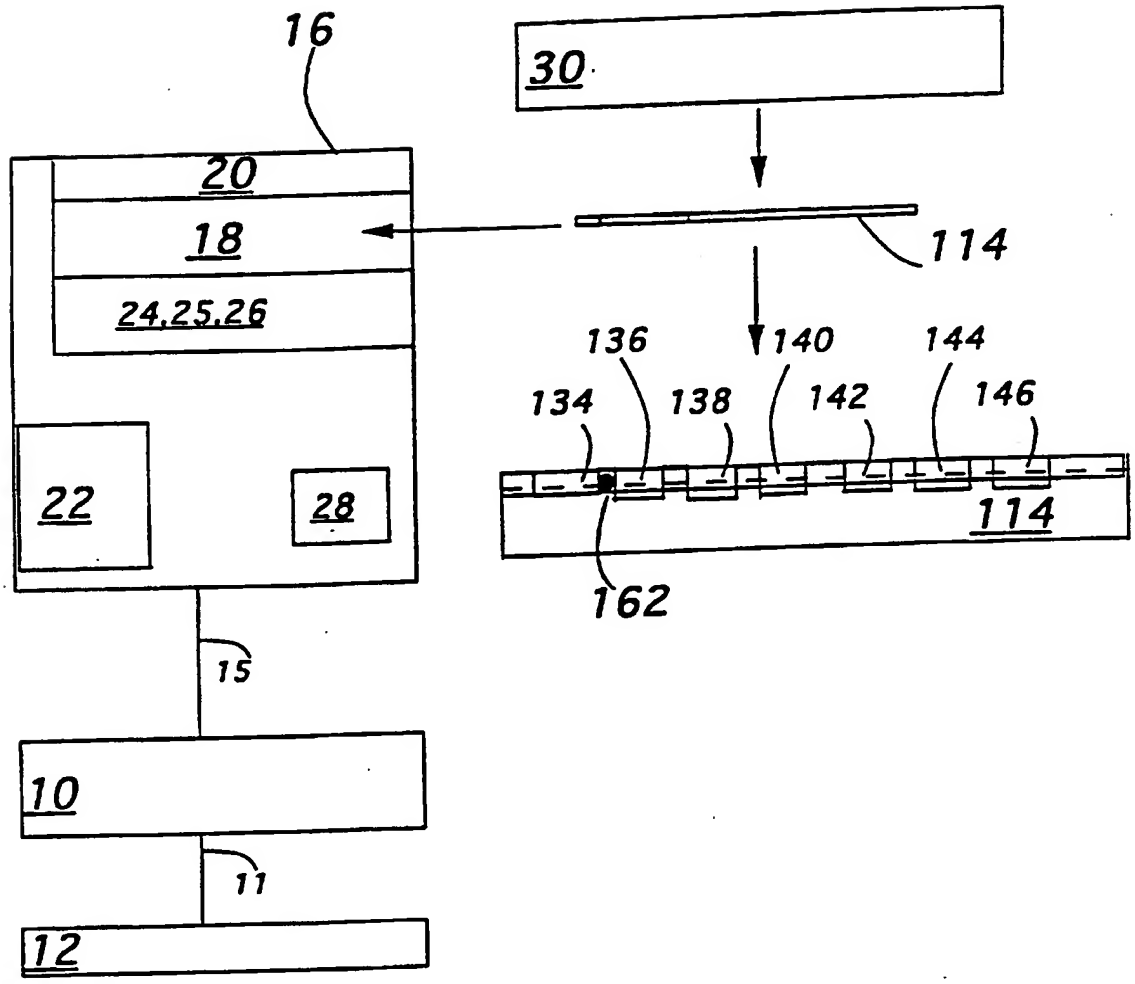
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Fig. 7A

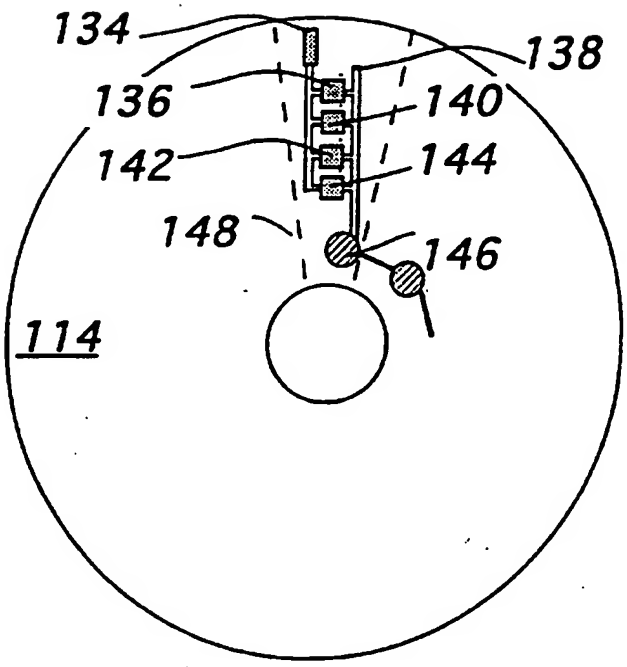


FIG. 7B

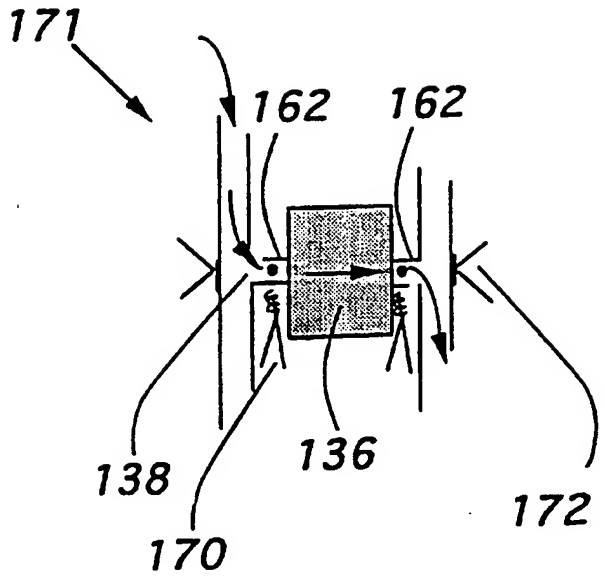
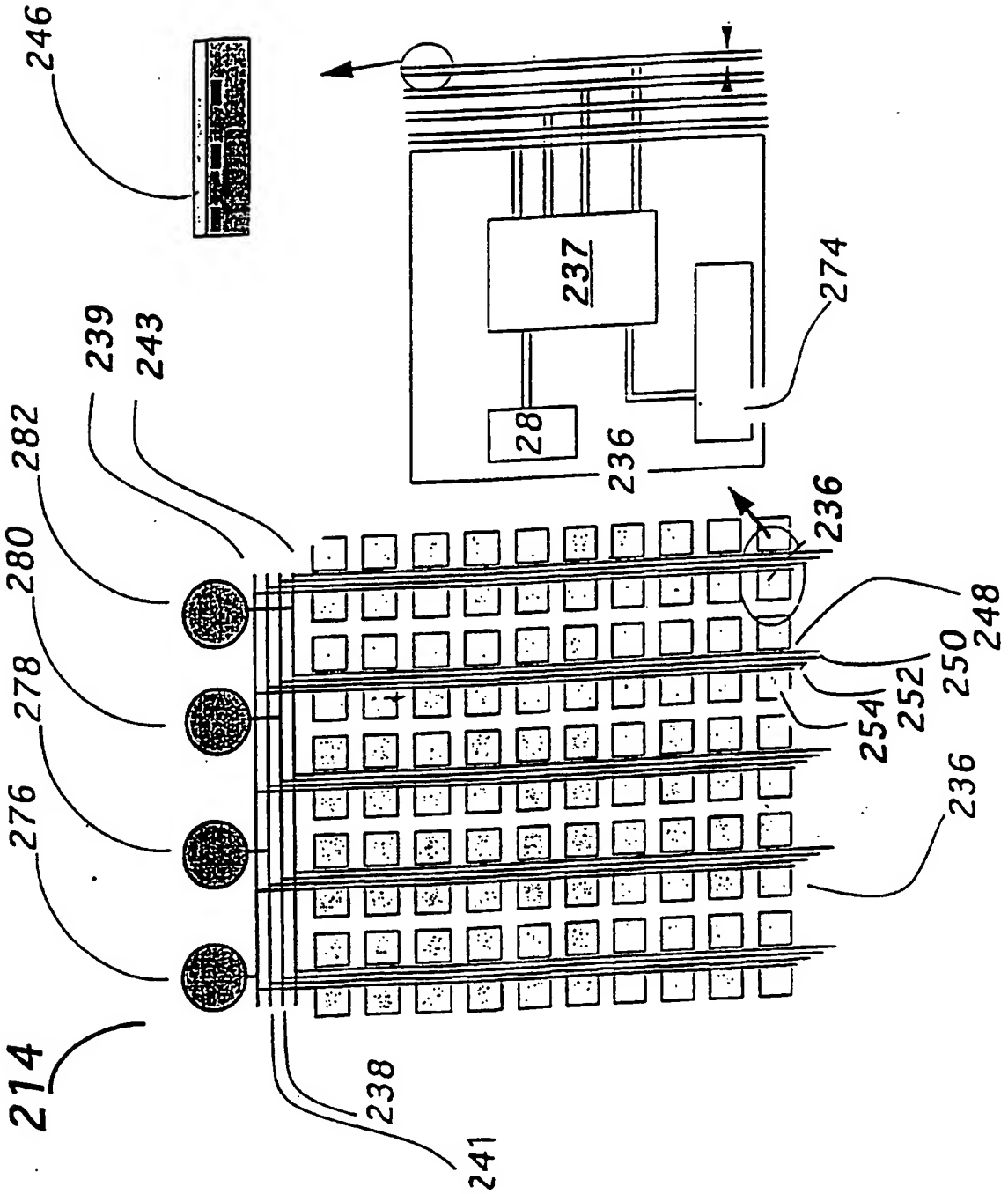


Fig. 7C

FIG. 8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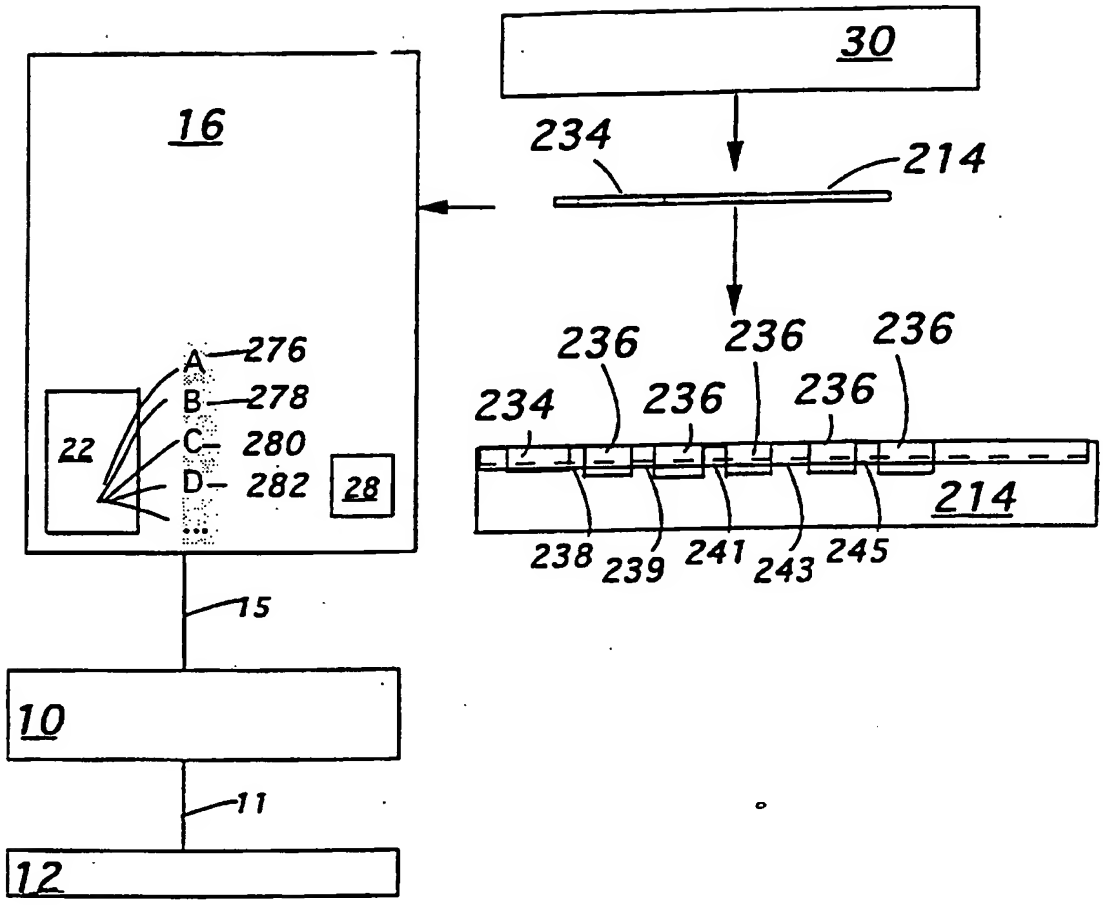
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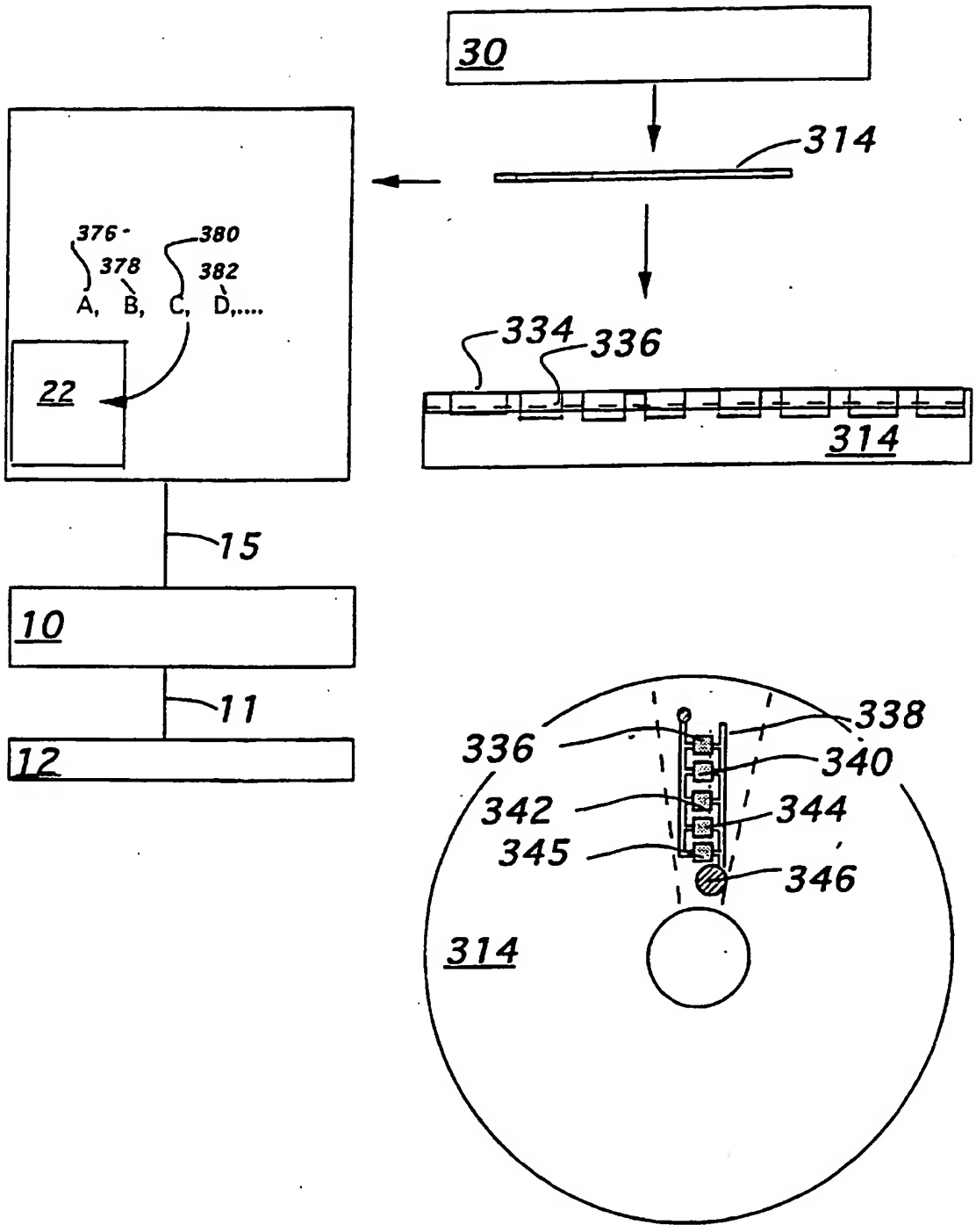
FIG. 9


30
314
376
380
378
382
A, B, C, D,....
22
334
336
314
15
10
11
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338
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342
344
345
346
314

FIG. 10

demonstrated by gel-shift assay. The results shown in Figures 6 and 7 demonstrate that hybrids without heterologous insert (i.e., triplex or quadruplex sequences) decay much faster than hybrids with completely homologous probes (controls), indicating that heterologous inserts are interacting with each other within the double D-loop.

!!! Example 1

Increased kinetic stability of triplex and quadraplex locks

In this example, the thermal stability of a triplex and quadraplex lock was evaluated, as compared to targeting polynucleotides lacking an anchoring sequence.

Three sets of complementary single stranded (css) probes were designed as follows. The target sequence was the 62 nucleotides from bases 667 to 723 of pBluescript II SK(-) (Figure 9A). The control reaction comprises two complementary single stranded nucleic acids (cssDNA) comprising these 62 bases and their complement. !!Targeting polynucleotides comprising the quadruplex forming lock 5-TTGGGGTTGGGGTT are shown in Figure 9C. Targeting polynucleotides comprising triplex forming locks are:

5-CCTCGAGGTCGACGGTATCGATAAGCTTGAT CCCTCCTCCCTCCCCTAATACCCCACCCA CCACCC ATCGAATTCCTGCAGCCCGGGGGATCCACTA-3 (SEQ ID NO:##) and

5-TAGTGGATCCCCCGGGCTGCAGGAATTCGAT GGGTGGTGGGTGGGGTATTAGGGGAGGGA GGAGGG ATCAAGCTTATCGATACCGTCGACCTCGAGG-3 (SEQ ID NO:##). !!PANGENE: THERE IS A DISCREPANCY BETWEEN THE SEQUENCES FLANKING THE QUADRUPLEX, TRIPLEX, AND Z-DNA SEQUENCES. AS SHOWN IN FIGURE 9 3' FROM THE HINDIII SITE IS "GAT" AND 3' FROM THE ECORI SITE IS "GAT". THESE THREE BASES ARE NOT FOUND IN THE SEQUENCE SHOWN IN FIGURE 16.

The triplex forming sequence is shown in bold (Dayn et al., PNAS USA 89:11406 (1992)).

The targeting polynucleotides were obtained either by chemical synthesis or PCR of a pBluescript II SK(-) containing the quadruplex or triplex lock sequence clone into the *Hin*dIII-*Eco*RI sites (Figure 9 and Figure 16). Oligonucleotides and pBluescript II SK(-) purification, RecA coating of oligonucleotides, targeting reactions, and deproteinization of hybrids by SDS treatment were performed as described in Example 2.

Hybrids were linearized by *Sca*I or *Pvu*II r striction enzyme digestion. To compare the stability of the three hybrids (contr l, quadruplex, and tripex hybrids), they wer incubated at 65°C, n utral pH in 6 mM Mg^{2+}, 50 mM Na^{+}, 50 mM K^{+}. At various time points, hybrid d cay was

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14589

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,376,252 (EKSTROM ET AL.) 27 December 1994	

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14589

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US, A, 4,963,498 (HILLMAN ET AL.) 16 October 1990, col. 2, ln. 39-51; col. 4, ln. 17-27; col. 8, ln. 56 - col. 9, ln. 20; col. 11, ln. 1-35; and col. 8, ln. 4-21.	20 and 21 ——— 5, 23, and 27
Y	US, A, 5,296,375 (KRICKA ET AL.) 22 MARCH 1994, col. 7, ln. 40-59; col. 7, ln. 13-18; col. 3, ln. 61-64; col. 3, ln. 65 - col. 4, ln.7; Fig. 4; col. 11, ln. 11-31; and claims 4, 15, and 21.	5, 7-10, and 23-27
Y	US, A, 5,262,127 (WISE ET AL.) 16 November 1993, col. 1, ln. 54-56; col. 2, ln. 46-56; claims 1, 2, and 4; col. 1, ln. 56-58; and col. 2, ln. 49-59.	25 and 26
Y	US, A, 5,304,487 (WILDING ET AL.) 19 April 1994, claim 1; abstract; col. 3, ln. 23-30; col. 3, ln. 44-47; col. 3, ln. 56- col.4 ln. 22; col. 4, ln. 25-29; Fig. 4; col. 5, ln. 58-62; claim 9; col. 8, ln. 1-7; claims 17 and 18; col. 6, ln. 49-50; claim 16; col. 9, ln. 42-48; col. 4, ln. 25-29; claims 19 and 20; col. 10, ln. 8-12; col. 10, ln. 46-54; col. 4, ln. 25-29; Table 1 (col. 4); Example 2 (col. 10-11); col. 8, ln. 20-45; col. 10, ln. 54-56; and col. 11, ln.19-21.	1-10 and 23-27
Y	WO, A1, 93/22058 (WILDING ET AL.) 11 November 1993, pages 6-11; and Fig. 3A, 10, 12, and 13.	11, 14, 16, 17, 20,21,23,24,25, and 26
A	US, A, 5,250,263 (MANZ) 05 October 1993	
A	US, A, 4,891,120 (SETHI ET AL.) 02 January 1990.	
A	US, A, 5,252,294 (KROY ET AL.) 12 October 1993.	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14589

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/50, 33/483, 35/00, 27/26; C12M 15/10
US CL : 422/68.1; 436/43; 935/87, 88; 204/454, 452, 601, 603
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/68.1; 436/43; 935/87, 88; 204/454, 452, 601, 603

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, INPADOC, JAPIO, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,180,480 (Manz) 19 January 1993, abstract; col. 2, ln. 64 - col. 3, ln. 9; and col. 3, ln. 37 - 67.	18
X — Y	US, A, 5,194,133 (CLARK ET AL.) 16 MARCH 1993, col. 2, ln. 24-31; col. 3, ln. 13-36; col. 4, ln. 54 - col. 5, ln. 34; Fig. 5; claims 1, 20, and 21; col. 5, ln. 41-59; col. 5, ln. 60 - col. 7, ln. 58; col. 2, ln. 36-68; and Fig. 1-5	1,4,6 ---------- 2, 3, 5, 7-10, 27-31
X,E — Y	US, A, 5,480,614 (KAMAHORI) 02 January 1996, col. 7, ln. 15 - col. 9, ln. 48; claims 1-3 and 7.	1-3 ---------- 8, 9, and 11-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 MARCH 1996	Date of mailing of the international search report 23 APR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Vandna Roy for Alexander Noguerola Telephone No. (703) 305-5686

Form PCT/ISA/210 (second sheet)(July 1992)*

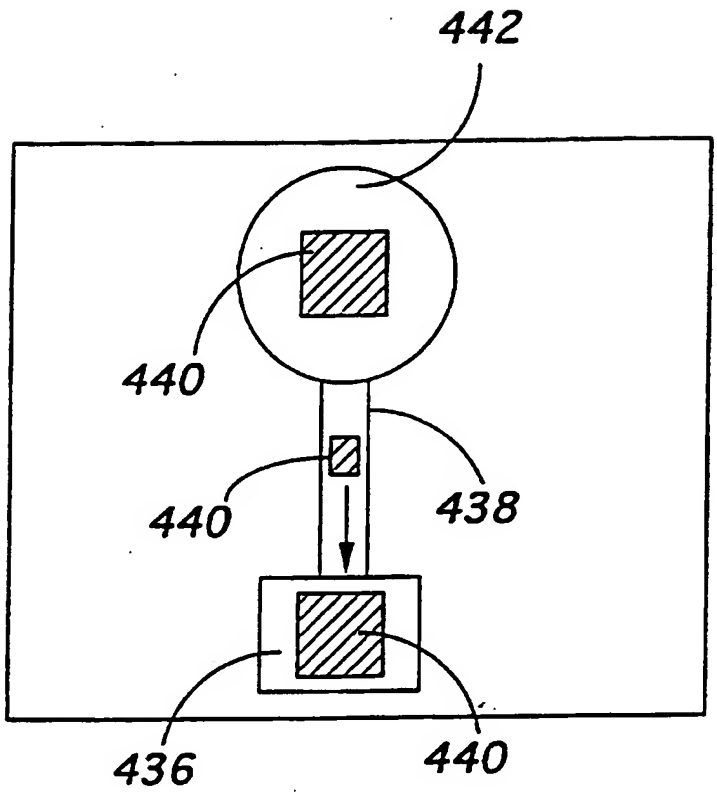

FIG. 12

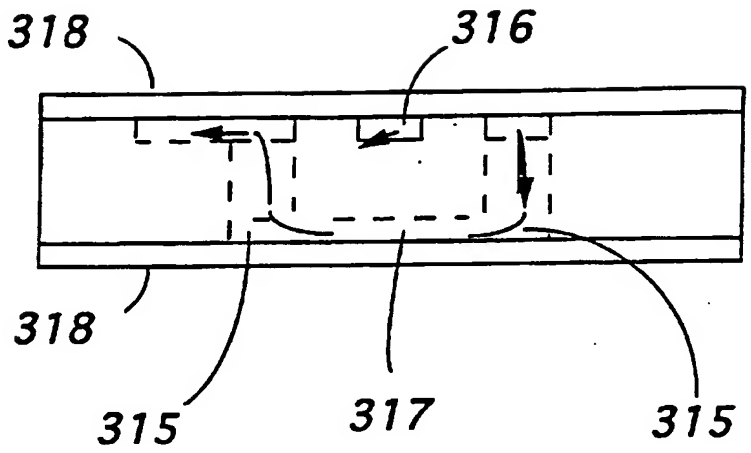

FIG. 11C

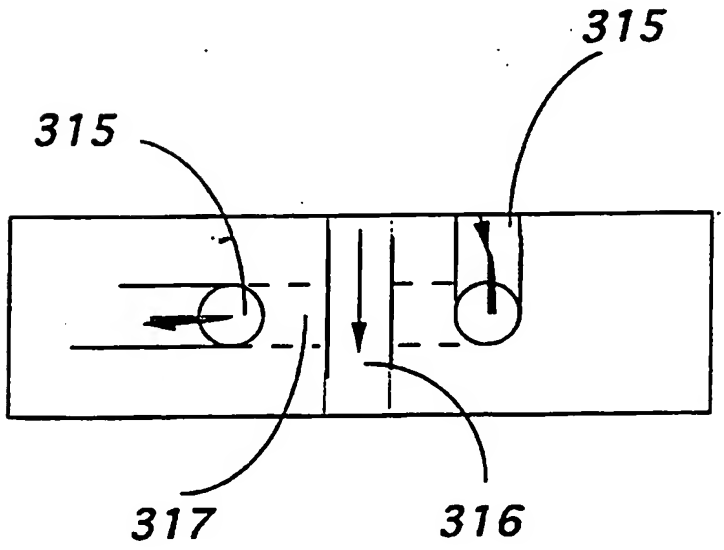

FIG. 11D

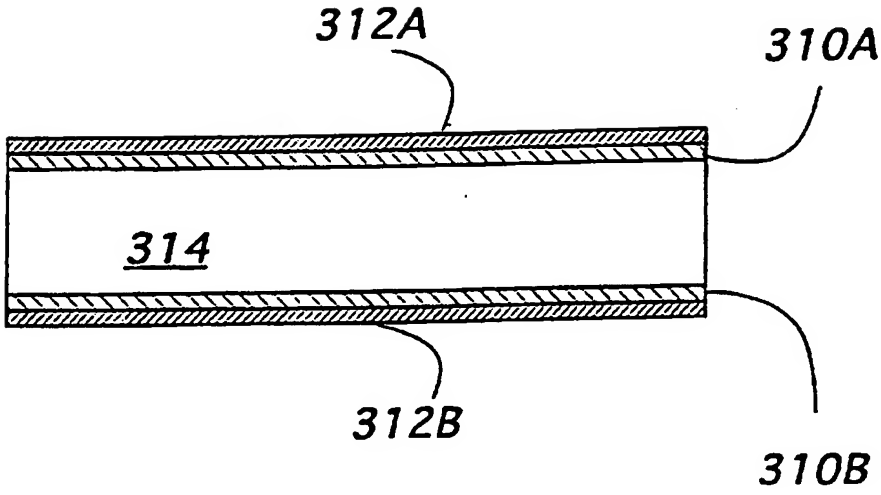

FIG. 11A

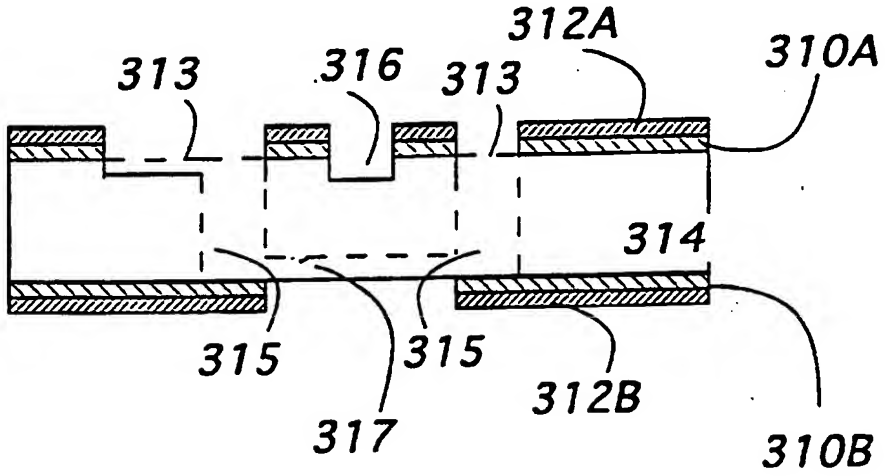

FIG. 11B

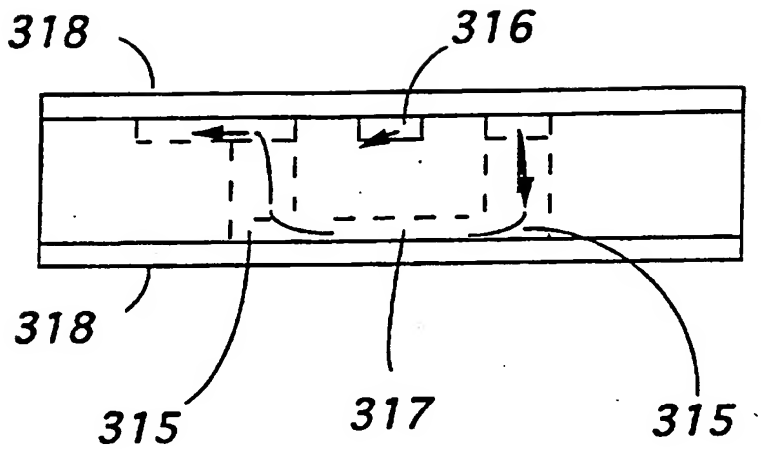

FIG. 11C

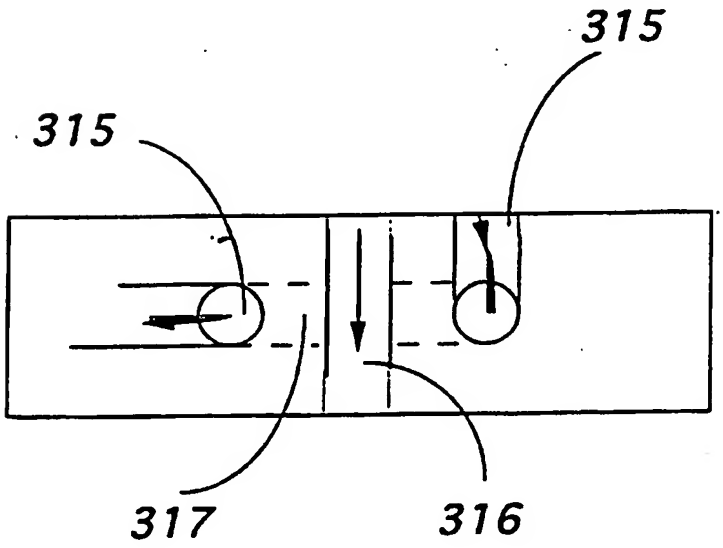

FIG. 11D

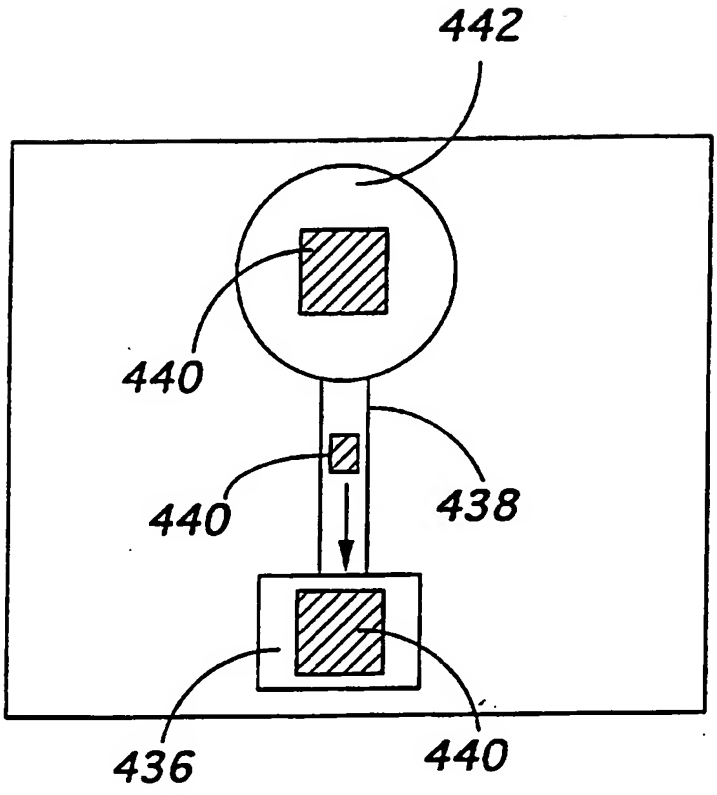

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14589

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/50, 33/483, 35/00, 27/26; C12M 15/10
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Vandna Roy for Alexander Noguerola Telephone No. (703) 305-5686

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14589

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Y	US, A, 5,304,487 (WILDING ET AL.) 19 April 1994, claim 1; abstract; col. 3, ln. 23-30; col. 3, ln. 44-47; col. 3, ln. 56- col.4 ln. 22; col. 4, ln. 25-29; Fig. 4; col. 5, ln. 58-62; claim 9; col. 8, ln. 1-7; claims 17 and 18; col. 6, ln. 49-50; claim 16; col. 9, ln. 42-48; col. 4, ln. 25-29; claims 19 and 20; col. 10, ln. 8-12; col. 10, ln. 46-54; col. 4, ln. 25-29; Table 1 (col. 4); Example 2 (col. 10-11); col. 8, ln. 20-45; col. 10, ln. 54-56; and col. 11, ln.19-21.	1-10 and 23-27
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